PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 10 November 2000 (10.11.00)	
International application No. PCT/US00/07959	**Applicant's or agent's file reference** 4239-54282
International filing date (day/month/year) 23 March 2000 (23.03.00)	**Priority date** (day/month/year) 24 March 1999 (24.03.99)
Applicant SHEARER, Gene, M. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 October 2000 (17.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia TEFY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

US0007959

936537

Copy for the Elected Office (EO/US)

PCT/US00/0795

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

NOONAN, William, D.
Klarquist, Sparkman, Campbell
Leigh & Whinston, LLP
Suite 1600 - One World Trade Center
121 S.W. Salmon Street
Portland, OR 97204
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
04 October 2001 (04.10.01)

Applicant's or agent's file reference
4239-54282

IMPORTANT NOTIFICATION

International application No.
PCT/US00/07959

International filing date (day/month/year)
23 March 2000 (23.03.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
ZOU, Jian-Ping
263 Congressional Lane
Rockville, MD 20852-5318
United States of America

State of Nationality: CN
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
ZUO, Jian-Ping
263 Congressional Lane
Rockville, MD 20852-5318
United States of America

State of Nationality: CN
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:
Correction of inventor's name.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
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Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004347119

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
4239-54282		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 07959	23/03/2000	24/03/1999
Applicant		
THE GOVERNMENT OF THE UNITED STATES OF AMERICA,...		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-17,20-25,28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 1-17,20-25,28

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

International Application No
/US 00/07959

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/14 A61P37/06 A61P21/00 A61P19/02 A61P3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, PASCAL, LIFESCIENCES, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 155 433 A (FONTANA ADRIANO) 25 September 1985 (1985-09-25) page 1, line 1 -page 4, line 25	1-33
X	EP 0 159 289 A (SANDOZ AG ;SANDOZ AG (DE); SANDOZ AG (AT)) 23 October 1985 (1985-10-23) page 1, line 1 -page 5, line 6	1-33

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 October 2000	26/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Rempp, G

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X,P	JIANG-PING ZOU ET AL.: "Human Glioma-Induced Immunosuppression Involves Soluble Factor(s) That Alters Monocyte Cytokine Profile and Surface Markers" JOURNAL OF IMMUNOLOGY., vol. 162, 1999, pages 4882-4892, XP002149737 THE WILLIAMS AND WILKINS CO. BALTIMORE., US ISSN: 0022-1767 the whole document ---	1-33
X,P	LORRI A. MORFORD ET AL. : "Apoptotic elimination of peripheral T lymphocytes in patients with primary intracranial tumors" JOURNAL OF NEUROSURGERY., vol. 91, no. 6, December 1999 (1999-12), pages 935-946, XP000952674 XX, XX ISSN: 0022-3085 the whole document -----	1-33

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
US 00/07959

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0155433 A	25-09-1985	AT 75778 T	15-05-1992
		AU 587973 B	07-09-1989
		AU 4157685 A	01-11-1985
		DE 3585968 A	11-06-1992
		DK 539285 A	21-11-1985
		WO 8504421 A	10-10-1985
		EP 0159289 A	23-10-1985
		IE 58821 B	17-11-1993
		IL 74680 A	30-11-1988
		JP 6080080 B	12-10-1994
		JP 61501514 T	24-07-1986
		NZ 211525 A	25-06-1991
		US 5095095 A	10-03-1992
		ZA 8501412 D	26-11-1986
		ZA 8502194 A	26-11-1986
EP 0159289 A	23-10-1985	EP 0155433 A	25-09-1985
		AT 75778 T	15-05-1992
		AU 587973 B	07-09-1989
		AU 4157685 A	01-11-1985
		DE 3585968 A	11-06-1992
		DK 539285 A	21-11-1985
		WO 8504421 A	10-10-1985
		IE 58821 B	17-11-1993
		IL 74680 A	30-11-1988
		JP 6080080 B	12-10-1994
		JP 61501514 T	24-07-1986
		NZ 211525 A	25-06-1991
		US 5095095 A	10-03-1992
		ZA 8501412 D	26-11-1986
		ZA 8502194 A	26-11-1986

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 September 2000 (28.09.2000)

PCT

(10) International Publication Number
WO 00/56356 A3

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(21) International Application Number: PCT/US00/07959

(22) International Filing Date: 23 March 2000 (23.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/125,996 24 March 1999 (24.03.1999) US

(71) Applicant *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES, THE NATIONAL INSTITUTES OF H EALTH** [US/US]; Office of Technology Transfer, Suite #325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

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(75) Inventors/Applicants *(for US only)*: **SHEARER, Gene, M.** [US/US]; 5512 Glenwood Road, Bethesda, MD 20817 (US). **ZOU, Jian-Ping** [CN/US]; 263 Congressional Lane, Rockville, MD 20852-5318 (US). **COLIGAN, John, E.** [US/US]; 10913 Broad Green Terrace, Potomac, MD 20854 (US). **CHOUGNET, Claire** [FR/US]; 2129 N. St. #4, Washington, DC 20037 (US).

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with amended claims and statement*

(88) Date of publication of the international search report:
25 January 2001

Date of publication of the amended claims and statement:
16 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: INDUCTION OF ANTIGEN-SPECIFIC UNRESPONSIVENESS BY GLIOBLASTOMA CULTURE SUPERNATANTS (GCS)

(57) Abstract: The present invention concerns methods of specifically inhibiting an immune response of a subject to one or more selected antigens using an immunosuppressive composition derived from a glioblastoma cell line. The method steps include obtaining a population of antigen presenting cells (APCs); loading the APC population with specific antigens (in auto-immune diseases) or using donor APCs (for transplantation); incubating the APC population with the immunosuppressive composition; and introducing the incubated cells into the subject being treated. The APCs can be monocytes, macrophages, or dendritic cells. This method causes specific inhibition of the immune response because it induces apoptosis and/or anergy in the subject's T cells specific for antigens present on the APCs, but does not affect the immune response to antigens not present on the APC surfaces. One particular embodiment of the present method is the specific inhibition of a transplant recipient's immune reaction to antigens present on the allogenic graft. A second particular embodiment of the present method is the specific inhibition of the immune response to an autoantigenic protein by a subject suffering from an autoimmune disease.

WO 00/56356 A3

AMENDED CLAIMS

[received by the International Bureau on 23 December 2000 (23.12.00);
original claims 31 and 32 renumbered 30 and 31;
remaining claims unchanged (6 pages)]

1. A method of specifically inhibiting an immune response to one or more selected antigens comprising:

exposing purified or isolated antigen presenting cells (APCs) that present an antigen against which selective inhibition of an immune response is desired to an immunosuppressive composition comprising one or more factors secreted by a glioblastoma cell.

2. The method of claim 1, further comprising:

introducing the purified or isolated APCs that have been exposed to the immunosuppressive composition into a subject in whom a reduced immune response to the antigen is desired, in a therapeutically effective amount sufficient to selectively inhibit the immune response of the subject to the antigen.

3. The method of claim 1, wherein the purified or isolated APCs are obtained from a transplant donor, and wherein the APCs express a transplant antigen against which specific inhibition of the immune response is desired.

4. The method of claim 1, wherein the APCs are obtained from a subject, wherein the APCs present an autoantigenic antigen against which specific inhibition of the immune response is desired.

5. The method of claim 4, wherein the purified or isolated APCs are incubated with an autoantigenic peptide, in an amount effective to cause the APCs to present the autoantigenic peptide.

6. The method of claim 1, wherein the method specifically inhibits the immune response by inducing apoptosis and/or anergy in T cells specific for the antigen.

7. The method of claim 2, wherein the APCs are obtained from a donor other than the subject, and the selected antigens are donor-specific antigens present on an allogenic graft.

8. The method of claim 7, wherein the APCs are obtained from a donor of an allogenic graft that is transplanted to the subject, and the introducing step comprises administering a sufficient dose of the exposed APCs to the subject to specifically inhibit the subject's immune response against an antigen presented by the APCs of the donor.

9. The method of claim 1 wherein the antigen presented by the APCs is an autoantigenic protein of an autoimmune disease.

10. The method of claim 9, wherein the purified or isolated APCs are obtained from a subject suffering from an autoimmune disease, and the isolated or purified APCs are repetitively exposed to one or more peptide fragments of the autoantigenic protein of the autoimmune disease, and the introducing step comprises administering a therapeutically effective amount of the exposed APCs to the subject.

11. The method of claim 10, wherein the autoimmune disease is selected from the group consisting of multiple sclerosis (MS), rheumatoid arthritis (RA), myasthenia gravis (MG), systemic lupus erythematosus (SLE), and insulin dependent diabetes mellitus (IDDM).

12. The method of claim 10, wherein the autoantigenic protein is selected from the group consisting of myelin basic protein (MBP), type II collagen, acetyl choline receptor (AcChoR), nuclear proteins, and pancreatic islet cell antigens.

13. The method of claim 1, wherein the APCs are selected from the group consisting of monocytes, macrophages, and dendritic cells.

14. The method of claim 13, wherein the APCs comprise monocytes.

15. The method of claim 8, wherein the APCs comprise monocytes isolated or purified from the donor's blood.

16. The method of claim 9, wherein the APCs comprise monocytes isolated or purified from the subject's blood.

17. The method of claim 1, wherein the glioblastoma cell is selected from the group consisting of SNB 19, U251 A172, A1207, A1235, A2781, U87 MG, U138 MG and U373 MG.

18. A purified immunosuppressive composition for use in selectively reducing an immune response to one or more selected antigens in a subject, the composition comprising one or more factors secreted by a glioblastoma cell that have the following characteristics:

a) incubation of the composition with APCs presenting an antigen, and subsequent exposure of the incubated APCs to T cells specific for the antigen, induces the T cells to undergo anergy or apoptosis;

b) a molecular weight greater than about 40 kDa;

c) ability to bind to anion, but not cation exchange columns;

d) maintain an ability to induce T cells to undergo anergy or apoptosis under the conditions of a) within the pH range of 2 to 11, following heat exposure up to about 56° C, and following immunoprecipitation of TGF- ß1, TGF- ß2, TGF- ß3, IL-6, calcitonin gene related peptide (CGRP), and M-CSF from the composition; and

e) loses the ability to induce T cells to undergo anergy or apoptosis under the conditions of a) following heat exposure above 56° C, or after exposure to trypsin.

19. The immunosuppressive composition of claim 18, wherein incubation of the composition with an effective amount of monocytes, dendrites, and B cells causes effects comprising the following:

a) decreased expression of MHC class II antigens and CD 80/86 on the surface of the monocytes and the dendrites, but no effect on the expression of MHC class II antigens and CD 80/86 on the B cells;

b) increased expression of IL-10 in monocytes and dendrites; and

c) decreased the expression of IL-12 in monocytes and dendrites.

20. A method for enhancing tolerance in a host mammal to an allogenic donor graft, comprising:

exposing APCs obtained from a donor mammal to a therapeutically effective amount of a composition secreted by a glioblastoma cell, wherein the composition is effective to induce the APCs obtained from the donor mammal to secrete one or more factors that selectively inhibit clonal proliferation of a T cell that specifically recognizes an allogenic antigen presented by the APCs obtained from the donor mammal; and

administering a therapeutically effective dose of the APCs obtained from a donor mammal that have been exposed to the therapeutically effective amount of the composition secreted by the glioblastoma cell to the host mammal to inhibit recognition of the allogenic antigen by the host mammal by inhibiting the clonal proliferation of the T cell of the host mammal in response to presentation of the allogenic antigen by the APCs.

21. The method of claim 20, wherein the allogenic antigen is an antigen from the allogenic donor graft.

22. The method of claim 20, wherein obtaining the donor mammalian APCs comprises specifically isolating or purifying APCs that recognize the allogenic antigen in the donor graft.

23. The method of claim 22, wherein the APCs are obtained from a source selected from the group consisting of an organ, tissue, bone marrow and blood of the donor mammal.

24. A method for enhancing tolerance in a host mammal to an autoantigen, comprising:

obtaining APCs from the host mammal, wherein the APCs present an antigen to T cells which recognize the antigen and undergo a specific clonal proliferation to induce an immune response against the antigen;

culturing the APCs ex vivo in an effective amount of a composition secreted by a glioblastoma cell in an amount effective to induce the APCs to secrete one or more factors that selectively inhibit the specific clonal proliferation of the T cells that recognize the autoantigen presented by the APCs; and

administering a therapeutically sufficient dose of the APCs to the host mammal to inhibit recognition of the autoantigen by the host mammal.

25. The method of claim 2, wherein introducing the APCs into the subject comprises administering the APCs by a route selected from the group consisting of intravenous, subcutaneous, intramuscular, and intraperitoneal administration.

26. The composition of claim 18 for use as a medicament.

27. The composition of clam 18 for use in a method of treating an immune mediated disease, comprising administering the composition to the subject.

28. A method of inhibiting an immune response to one more selected antigens comprising contacting an APC with the composition of claim 18.

29. A method of making an immunosuppressive composition for use in suppressing an immune response to an antigen, comprising incubating a supernatant harvested from a glioblastoma cell culture and the antigen with an

APC, thereby producing an immunosuppressive composition that includes the APC.

30. The method of claim 29, further comprising combining the immunosuppressive composition with a pharmaceutical carrier.

31. The composition obtained by the method of claim 29.

32. The method of claim 29, further comprising purifying the APC to produce a substantially pure APC composition prior to incubating with the glioblastoma cell culture supernatant.

33. The method of claim 29, further comprising purifying the APC to produce a substantially pure APC composition after incubating with the glioblastoma cell culture supernatant.

STATEMENT UNDER ARTICLE 19

Claims 1-33 were pending in the present application. The search of claims 1-17, 20-25, and 28, directed to a method of treatment of the human/animal body, was carried out based on the alleged effects of the compound/composition. Category X and X, P documents were cited as relevant to claims 1-33.

EP 0 155 433 to Fontana and EP 0 159 289 to Sandoz were cited as Category X documents as applied to claims 1-33. Neither EP 0 155 433 nor EP 0 159 289 (herein the "EP references") disclose a method or a composition for inhibiting an immune response by exposing isolated or purified antigen presenting cells (APCs) that present an antigen, against which selective inhibition of an immune response is desired, to an immunosuppressive composition having one or more factors secreted by a glioblastoma cell.

Although T cell proliferation and IL-2 production were known to be defective in glioma patients and in cultures of PBMC exposed to glioblastoma cell supernatants, it was not known that these T cell defects have their origin in alternation of APC function. Therefore, the discovery that exposing *isolated or purified APCs* to an immunosuppressive composition secreted by a glioblastoma cell can be used to *specifically* inhibit an immune response against antigen(s) presented on the APCs is both novel and inventive. In turn, it would not have been obvious that exposure of the purified or isolated APCs to the composition could be used to treat graft rejection or autoimmune disorders.

Claims 1-17 and 20-25

Claims 1-17 and 25 of the present application are directed to a method of inhibiting an immune response to one or more selected antigens. The method generally involves exposing purified or isolated APCs, which present an antigen against which selective inhibition of an immune response is desired, to an immunosuppressive composition containing one or more factors secreted by a glioblastoma cell. Although the EP references disclose a 97 kD factor obtained from a glioblastoma supernatant which inhibits IL-2 dependent T-cell mechanisms, there is no teaching or suggestion that one or more factors secreted by a glioblastoma cell can be incubated with APCs, in order to *selectively* inhibit an immune response against the antigen(s) presented by the APC.

In one embodiment of the present invention, the APCs are exposed to the immunosuppressive composition *ex vivo*. This method results in the inhibition of the immune

response against the antigen(s) presented by the APC, not all antigens present in a subject. This selective inhibition provides a superior result to that taught by the EP references, which at most teach the administration of the immunosuppressive composition to the patient, not exposing the composition to isolated or purified APCs. Administration of the immunosuppressive composition to the patient will not likely produce a selective inhibition of the immune response. Instead, the patient's immune response would be indiscriminately inhibited, subjecting the patient to the risk of generalized immunosuppression and infection.

Claims 20-24 of the present application are directed to methods of enhancing tolerance in a host mammal to an allogenic donor graft or autoantigen. The method of enhancing tolerance in a host mammal to an allogenic donor graft involves exposing APCs obtained from a *donor* mammal to a composition secreted by a glioblastoma cell, and administering the exposed APCs to the host mammal to inhibit the host's immune response to the donor's allogenic antigen.

The EP references state that the disclosed immunosuppressant factor obtained from a glioblastoma supernatant can be "used in connection with transplant operations to prevent rejection" and to treat "diseases where suppression of the body's immune systems is indicated . . . [such as] auto-immune diseases" (page 20 EP 0 155 433 and page 23 EP 0 159 289). However, there is no teaching or suggestion that the tolerance in a *host* to an allogenic donor graft can be enhanced by exposing APCs obtained from a *donor* to a immunosuppressive composition secreted by a glioblastoma cell, and administering the exposed APCs to the host to inhibit the host's immune response to the donor's allogenic antigen. In addition, there is no teaching or suggestion that the tolerance of a host autoantigen can be enhanced by *ex vivo* exposure of APCs, obtained from the host, to a composition secreted by a glioblastoma cell, and administering the exposed APCs to the host to inhibit the host's immune response to the host's autoantigen.

Because claims 1-17 and 20-25 are directed to a previously unidentified method for *selective* inhibition of an immune response, the claims are novel and inventive in view of the prior art, and define patentable subject matter.

Claims 18, 19 and 26-33

Claims 18, 19 and 26-33 of the present application are directed to an immunosuppressive composition for *selectively* reducing an immune response in a subject,

and methods of using and making the composition. Although the EP references disclose an immunosuppressive factor obtained from a glioblastoma supernatant, there is no teaching or suggestion that one or more factors secreted by a glioblastoma cell can be used to *selectively* inhibit an immune response against an antigen presented by an APC. Instead, the administration of the composition to a subject in those references would provide a generalized inhibitory effect on APCs of the subject, which would result in non-selective inhibition of the immune response, and generalized (undesired) immunosuppression. As discussed above, the *selective* inhibition provided by the present application provides a superior result to compositions disclosed in the prior art, which result in a general inhibition of the immune response.

Because claims 8, 19 and 26-33 are directed to a previously unidentified immunosuppressive composition for *selectively* reducing an immune response in a subject, the claims are novel and inventive in view of the prior art, and define patentable subject matter.

In conclusion, the cited art of EP 0 155 433 to Fontana and EP 0 159 289 to Sandoz fails to disclose or suggest a method of exposing *isolated or purified APCs* to an immunosuppressive composition secreted by a glioblastoma cell to *specifically* inhibit an immune response against an antigen presented on the APCs, or compositions for such a method. The claims therefore define patentable subject matter.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 September 2000 (28.09.2000)

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60/125,996 24 March 1999 (24.03.1999) US

(71) Applicant *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES, THE NATIONAL INSTITUTES OF H EALTH** [US/US]; Office of Technology Transfer, Suite #325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SHEARER, Gene, M.** [US/US]; 5512 Glenwood Road, Bethesda, MD 20817 (US). **ZOU, Jian-Ping** [CN/US]; 263 Congressional Lane, Rockville, MD 20852-5318 (US). **COLIGAN, John, E.** [US/US]; 10913 Broad Green Terrace, Potomac, MD 20854 (US). **CHOUGNET, Claire** [FR/US]; 2129 N. St. #4, Washington, DC 20037 (US).

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
25 January 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/56356 A3

(54) Title: INDUCTION OF ANTIGEN-SPECIFIC UNRESPONSIVENESS BY GLIOBLASTOMA CULTURE SUPERNATANTS (GCS)

(57) Abstract: The present invention concerns methods of specifically inhibiting an immune response of a subject to one or more selected antigens using an immunosuppressive composition derived from a glioblastoma cell line. The method steps include obtaining a population of antigen presenting cells (APCs); loading the APC population with specific antigens (in auto-immune diseases) or using donor APCs (for transplantation); incubating the APC population with the immunosuppressive composition; and introducing the incubated cells into the subject being treated. The APCs can be monocytes, macrophages, or dendritic cells. This method causes specific inhibition of the immune response because it induces apoptosis and/or anergy in the subject's T cells specific for antigens present on the APCs, but does not affect the immune response to antigens not present on the APC surfaces. One particular embodiment of the present method is the specific inhibition of a transplant recipient's immune reaction to antigens present on the allogenic graft. A second particular embodiment of the present method is the specific inhibition of the immune response to an autoantigenic protein by a subject suffering from an autoimmune disease.

INTERNATIONAL SEARCH REPORT

Int[redacted]l Application No
PCT/US 00/07959

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/14 A61P37/06 A61P21/00 A61P19/02 A61P3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, PASCAL, LIFESCIENCES, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 155 433 A (FONTANA ADRIANO) 25 September 1985 (1985-09-25) page 1, line 1 -page 4, line 25	1-33
X	EP 0 159 289 A (SANDOZ AG ;SANDOZ AG (DE); SANDOZ AG (AT)) 23 October 1985 (1985-10-23) page 1, line 1 -page 5, line 6	1-33

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 October 2000	26/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Rempp, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In. Application No
PCT/US 00/07959

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X,P	JIANG-PING ZOU ET AL.: "Human Glioma-Induced Immunosuppression Involves Soluble Factor(s) That Alters Monocyte Cytokine Profile and Surface Markers" JOURNAL OF IMMUNOLOGY., vol. 162, 1999, pages 4882-4892, XP002149737 THE WILLIAMS AND WILKINS CO. BALTIMORE., US ISSN: 0022-1767 the whole document ---	1-33
X,P	LORRI A. MORFORD ET AL. : "Apoptotic elimination of peripheral T lymphocytes in patients with primary intracranial tumors" JOURNAL OF NEUROSURGERY., vol. 91, no. 6, December 1999 (1999-12), pages 935-946, XP000952674 XX, XX ISSN: 0022-3085 the whole document -----	1-33

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-17,20-25,28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 1-17,20-25,28

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[redacted]l Application No
PCT/US 00/07959

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0155433 A	25-09-1985	AT 75778 T	15-05-1992
		AU 587973 B	07-09-1989
		AU 4157685 A	01-11-1985
		DE 3585968 A	11-06-1992
		DK 539285 A	21-11-1985
		WO 8504421 A	10-10-1985
		EP 0159289 A	23-10-1985
		IE 58821 B	17-11-1993
		IL 74680 A	30-11-1988
		JP 6080080 B	12-10-1994
		JP 61501514 T	24-07-1986
		NZ 211525 A	25-06-1991
		US 5095095 A	10-03-1992
		ZA 8501412 D	26-11-1986
		ZA 8502194 A	26-11-1986
EP 0159289 A	23-10-1985	EP 0155433 A	25-09-1985
		AT 75778 T	15-05-1992
		AU 587973 B	07-09-1989
		AU 4157685 A	01-11-1985
		DE 3585968 A	11-06-1992
		DK 539285 A	21-11-1985
		WO 8504421 A	10-10-1985
		IE 58821 B	17-11-1993
		IL 74680 A	30-11-1988
		JP 6080080 B	12-10-1994
		JP 61501514 T	24-07-1986
		NZ 211525 A	25-06-1991
		US 5095095 A	10-03-1992
		ZA 8501412 D	26-11-1986
		ZA 8502194 A	26-11-1986

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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PATENT COOPERATION TREATY

PCT

REC'D 30 MAY 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
4239-54282		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/07959	23/03/2000	24/03/1999
International Patent Classification (IPC) or national classification and IPC A61K39/00		
Applicant THE GOVERNMENT OF THE UNITED STATES OF AMERICA,...		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/10/2000	28.05.2001
Name and mailing address of the international preliminary examining authority: *European Patent Office* D-80298 Munich *Tel. +49 89 2399 - 0 Tx: 523656 epmu d* Fax: +49 89 2399 - 4465	Authorized officer Jacques, P Telephone No. +49 89 2399 8934

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/07959

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-42 as originally filed

Claims, No.:

1-33 as originally filed

Drawings, sheets:

1/13-13/13 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any ***nucleotide and/or amino acid sequence*** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-17, 20-25, 28.

because:

☒ the said international application, or the said claims Nos. 1-17, 20-25, 28 (with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17, 20-25, 28-33
	No:	Claims	18-19, 26-27
Inventive step (IS)	Yes:	Claims	1-17, 20-25, 28-33
	No:	Claims	18-19, 26-27
Industrial applicability (IA)	Yes:	Claims	18-19, 26-27, 29-33
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 1-17, 20-25 and 28 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).
   In this context, the said claims are considered to fall under the concept of methods of treatment of the human/animal body (see further point 9 under Item V).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: EP-A-0 155 433 (FONTANA ADRIANO) 25 September 1985 (1985-09-25)
   D2: EP-A-0 159 289 (SANDOZ AG ;SANDOZ AG (DE); SANDOZ AG (AT)) 23 October 1985 (1985-10-23)

2. The documents cited as a P-document in the International Search Report are not to be regarded as state of the art according to Article 33(2) PCT, as the date of priority claimed can be allowed for the relevant parts of the present application.

3. The subject-matter of independent claim 18 is not new in the sense of Article 33(2) PCT for the following reasons:
   Documents D1 and D2 disclose the production of purified supernatant from glioblastoma cell line, the said composition comprising a factor secreted by the glioblastoma cells, having a molecular weight of 97 000 (see page 1, lines 18-20 and page 8, lines 10-31). Moreover, all the other features of claim 18 (a), c), d) and e)) are functional features which do not further define the immunosuppressive composition in terms of technical features. As the compositions disclosed in D1 and D2 are supernatants from glioblastoma cell line, it is likely that they present the same

functional characteristics.
Thus, all the features of claim 18 are already disclosed from D1 and D2.

4. The same objection applies to dependent claim 19, as the said claim does not further define the composition in term of technical features, and to claims 26 and 27 as D1 and D2 disclose the use of the said composition in the treatment of diseases and conditions where suppression of the body's immune response is desired (page 20, lines 1-3 and page 23, lines 1-4 respectively).

5. As the particular combination of features of independent claim 1 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

6. Moreover, the subject-matter of the said claim involves an inventive step in the sense of Article 33(3) PCT for the following reasons:
The closest state of the art is considered to result from documents D1 and D2.
These documents disclose that cultures of glioblastoma cell lines secrete a factor that inhibits IL-2 dependent T-cell mechnisms.

The subject-matter of claim 1 is distinguished therefrom in that APCs that present an antigen are incubated with a composition comprising one or more factor secreted by a glioblastoma cell line.
The technical effect of this distinguishing feature results in a selective inhibition of the immune response to the antigen carried by the APCs.
The technical problem to be solved by the invention was therefore to provide a method for specifically inhibiting an immune response to a selected antigen.

The above mentioned technical problem has convincingly been solved by the discovery that the immunoregulatory effects of glioma culture supernatant on the inhibition of T-cell proliferation has its origin in APCs. The applicant has shown that the incubation of APCs with glioma culture supernatant causes specific inhibition of the immune response because it induces apoptosis and/or anergy in the subject's T cells specific for the antigens presented by the APCs, but does not affect the immune response to antigens not present on the APC surfaces.

As the said solution is not disclosed, nor suggested in the cited prior art, the subject-matter of claim 1 involves an inventive step in the sense of Article 33(3) PCT.

The same applies to dependent claims 2 to 17 and 25.

7. The same reasoning applies to independent claims 20, 24, 28 and 29 as the subject-matter of the said claims relates to different methods based on the same inventive concept, as mentioned under point 6.
Thus, the subject-matter of claims 20, 24, 28, 29, and their respective dependent claims, is new (Article 33(2) PCT) and involves an inventive step in the sense of Article 33(3) PCT.

8. The subject-matter of claim 32 relates to a composition defined in terms of the process of claim 29. The said process defines the composition in that it is an immunosuppressive composition that includes the APC.
As such particular combination of features is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

Moreover, the said claim involves an inventive step (Article 33(3) PCT) for the same reasons as mentioned under above point 6.

9. For the assessment of the present claims 1-17, 20-25 and 28 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art

disclosed in the documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

1. The subject-matter of claim 17 is directed to various cell lines. However, as no reference to accession numbers are given, it is not clear from the description if all of the said cell lines have been made available to the public in such a manner as to enable the invention to be carried out by a person skilled in the art. Therefore, the subject-matter of claim 17 does not fulfill the requirements of Article 5 PCT.

2. Claim 25, which is dependent on claim 2, and claims 26 and 27, dependent on claim 18, are not grouped together with the claims on which they are dependent (Rule 6.4 a), b); c); PCT-Guidelines C-III, 3.6).

09/936537

IN THE INTERNATIONAL BUREAU OF WIPO

PATENT COOPERATION TREATY
The International Bureau of WIPO
Attention: Catherine Humbert

In Re International Application of: THE GOVERNMENT OF THE UNITED STATES OF AMERICA REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES; NATIONAL INSTITUTES OF HEALTH

International Application No.: PCT/US00/07959

International Filing Date: 23 March 2000 (23.03.00)

For: INDUCTION OF ANTIGEN-SPECIFIC UNRESPONSIVENESS BY GLIOBLASTOMA CULTURE SUPERNATANTS (GCS)

Date: December 23, 2000

ARTICLE 19 AMENDMENT AND STATEMENT

International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
Switzerland

Via Facsimile (41-22) 740 14 35

These remarks accompany an Article 19 amendment in reply to the International search report dated 26 October 2000. A two-month period for response was set, making a response due on or before 27 December 2000.

Article 19 Amendment

The claims have been amended as shown in the attached annotated copy of the claims, wherein bracketing indicates a deletion and underlining indicates an addition. The original claims had no claim 30, but two claims numbered 32. Therefore, original claim 31 was renumbered and is now claim 30, and prior claim 32 was renumbered and is now claim 31.

Also enclosed are substitute pages 43-48 which provide a non-annotated copy of the amended claims.

Support for the claim amendments can be located in the specification on pages 4-6.

Statement

Claims 1-33 were pending in the present application. The search of claims 1-17, 20-25, and 28, directed to a method of treatment of the human/animal body, was carried out based on the alleged effects of the compound/composition. Category X and X, P documents were cited as relevant to claims 1-33.

EP 0 155 433 to Fontana and EP 0 159 289 to Sandoz were cited as Category X documents as applied to claims 1-33. Neither EP 0 155 433 nor EP 0 159 289 (herein the "EP references") disclose a method or a composition for inhibiting an immune response by exposing isolated or purified antigen presenting cells (APCs) that present an antigen, against which selective inhibition of an immune response is desired, to an immunosuppressive composition having one or more factors secreted by a glioblastoma cell.

Although T cell proliferation and IL-2 production were known to be defective in glioma patients and in cultures of PBMC exposed to glioblastoma cell supernatants, it was not known that these T cell defects have their origin in alternation of APC function. Therefore, the discovery that exposing *isolated or purified APCs* to an immunosuppressive composition secreted by a glioblastoma cell can be used to *specifically* inhibit an immune response against antigen(s) presented on the APCs is both novel and inventive. In turn, it would not have been obvious that exposure of the purified or isolated APCs to the composition could be used to treat graft rejection or autoimmune disorders.

Claims 1-17 and 20-25

Claims 1-17 and 25 of the present application are directed to a method of inhibiting an immune response to one or more selected antigens. The method generally involves exposing purified or isolated APCs, which present an antigen against which selective inhibition of an immune response is desired, to an immunosuppressive composition containing one or more factors secreted by a glioblastoma cell. Although the EP references disclose a 97 kD factor obtained from a glioblastoma supernatant which inhibits IL-2 dependent T-cell mechanisms, there is no teaching or suggestion that one or more factors secreted by a glioblastoma cell can be incubated with APCs, in order to *selectively* inhibit an immune response against the antigen(s) presented by the APC.

In one embodiment of the present invention, the APCs are exposed to the immunosuppressive composition *ex vivo*. This method results in the inhibition of the immune

response against the antigen(s) presented by the APC, not all antigens present in a subject. This selective inhibition provides a superior result to that taught by the EP references, which at most teach the administration of the immunosuppressive composition to the patient, not exposing the composition to isolated or purified APCs. Administration of the immunosuppressive composition to the patient will not likely produce a selective inhibition of the immune response. Instead, the patient's immune response would be indiscriminately inhibited, subjecting the patient to the risk of generalized immunosuppression and infection.

Claims 20-24 of the present application are directed to methods of enhancing tolerance in a host mammal to an allogenic donor graft or autoantigen. The method of enhancing tolerance in a host mammal to an allogenic donor graft involves exposing APCs obtained from a *donor* mammal to a composition secreted by a glioblastoma cell, and administering the exposed APCs to the host mammal to inhibit the host's immune response to the donor's allogenic antigen.

The EP references state that the disclosed immunosuppressant factor obtained from a glioblastoma supernatant can be "used in connection with transplant operations to prevent rejection" and to treat "diseases where suppression of the body's immune systems is indicated . . . [such as] auto-immune diseases" (page 20 EP 0 155 433 and page 23 EP 0 159 289). However, there is no teaching or suggestion that the tolerance in a *host* to an allogenic donor graft can be enhanced by exposing APCs obtained from a *donor* to a immunosuppressive composition secreted by a glioblastoma cell, and administering the exposed APCs to the host to inhibit the host's immune response to the donor's allogenic antigen. In addition, there is no teaching or suggestion that the tolerance of a host autoantigen can be enhanced by *ex vivo* exposure of APCs, obtained from the host, to a composition secreted by a glioblastoma cell, and administering the exposed APCs to the host to inhibit the host's immune response to the host's autoantigen.

Because claims 1-17 and 20-25 are directed to a previously unidentified method for *selective* inhibition of an immune response, the claims are novel and inventive in view of the prior art, and define patentable subject matter.

Claims 18, 19 and 26-33

Claims 18, 19 and 26-33 of the present application are directed to an immunosuppressive composition for *selectively* reducing an immune response in a subject,

and methods of using and making the composition. Although the EP references disclose an immunosuppressive factor obtained from a glioblastoma supernatant, there is no teaching or suggestion that one or more factors secreted by a glioblastoma cell can be used to *selectively* inhibit an immune response against an antigen presented by an APC. Instead, the administration of the composition to a subject in those references would provide a generalized inhibitory effect on APCs of the subject, which would result in non-selective inhibition of the immune response, and generalized (undesired) immunosuppression. As discussed above, the *selective* inhibition provided by the present application provides a superior result to compositions disclosed in the prior art, which result in a general inhibition of the immune response.

Because claims 8, 19 and 26-33 are directed to a previously unidentified immunosuppressive composition for *selectively* reducing an immune response in a subject, the claims are novel and inventive in view of the prior art, and define patentable subject matter.

In conclusion, the cited art of EP 0 155 433 to Fontana and EP 0 159 289 to Sandoz fails to disclose or suggest a method of exposing *isolated or purified APCs* to an immunosuppressive composition secreted by a glioblastoma cell to *specifically* inhibit an immune response against an antigen presented on the APCs, or compositions for such a method. The claims therefore define patentable subject matter.

Respectfully submitted,

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Annotated Copy of Claims Showing Additions and Deletions

We claim:

1. (Amended) A method of specifically inhibiting an immune response to one or more selected antigens comprising:

[a) providing antigen presenting cells (APCs) that present an antigen against which selective inhibition of an immune response is desired;]

[b)] [incubating the] exposing purified or isolated antigen presenting cells (APCs) that present an antigen against which selective inhibition of an immune response is desired [with] to an immunosuppressive composition comprising one or more factors secreted by a glioblastoma cell [line].

2. (Amended) The method of claim 1, further comprising

[c)] introducing the purified or isolated APCs that have been exposed to the immunosuppressive composition into a subject in [need of] whom a reduced immune response to the antigen is desired, in a therapeutically effective amount sufficient to selectively inhibit the immune response of the subject to the antigen.

3. (Amended) The method of claim 1, wherein [providing] the purified or isolated APCs [comprises obtaining APCs] are obtained from a transplant donor, and wherein the APCs express a transplant antigen against which specific inhibition of the immune response is desired.

4. (Amended) The method of claim 1, wherein [providing] the APCs [comprises obtaining APCs] are obtained from [the] a subject, wherein the APCs present an autoantigenic antigen against which specific inhibition of the immune response is desired.

5. (Amended) The method of claim [5] 4, wherein [providing] the purified or isolated APCs [comprises incubating the APCs] are incubated with an autoantigenic peptide[s], in an amount effective to cause the APCs to present the autoantigenic peptide.

6. (Amended) The method of claim 1, wherein the method specifically inhibits the immune response by inducing apoptosis and/or anergy in T cells specific for the [selected] antigen[s].

7. (Amended) The method of claim 2, wherein the APCs are obtained from a donor other than [a] the subject, and the selected antigens are donor-specific antigens present on an allogenic graft.

8. (Amended) The method of claim [2] 7, wherein the APCs are obtained from a donor of an allogenic graft that is transplanted to the subject, and the introducing step comprises administering a sufficient dose of the [incubated cells] exposed APCs to the subject to specifically inhibit the subject's immune response against an antigen presented by the APCs of the donor.

9. (Amended) The method of claim 1 wherein the [selected] antigen presented by the APCs is an autoantigenic protein of an autoimmune disease.

10. (Amended) The method of claim [8] 9, wherein the [providing step comprises isolating] purified or isolated APCs are obtained from a subject suffering from an autoimmune disease, and [repetitively exposing] the isolated or purified APCs are repetitively exposed to one or more peptide fragments of the autoantigenic protein of the autoimmune disease, and the introducing step comprises administering a therapeutically effective amount of the exposed APCs to the subject.

11. (Reiterated) The method of claim 10, wherein the autoimmune disease is selected from the group consisting of multiple sclerosis (MS), rheumatoid arthritis (RA), myasthenia gravis (MG), systemic lupus erythematosus (SLE), and insulin dependent diabetes mellitus (IDDM).

12. (Reiterated) The method of claim 10, wherein the autoantigenic protein is selected from the group consisting of myelin basic protein (MBP), type II collagen, acetyl choline receptor (AcChoR), nuclear proteins, and pancreatic islet cell antigens.

13. (Reiterated) The method of claim 1, wherein the APCs are selected from the group consisting of monocytes, macrophages, and dendritic cells.

14. (Reiterated) The method of claim 13, wherein the APCs comprise monocytes.

15. (Amended) The method of claim 8, wherein the APCs comprise monocytes isolated or purified from the donor's blood.

16. (Amended) The method of claim 9, wherein the APCs comprise monocytes isolated or purified from the subject's blood.

17. (Amended) The method of claim 1, wherein the glioblastoma [line] cell is selected from the group consisting of SNB 19, U251 A172, A1207, A1235, A2781, U87 MG, U138 MG and U373 MG.

18. (Amended) A purified immunosuppressive composition for [the reduction of] use in selectively reducing an immune response to one or more selected antigens in a subject, the composition comprising one or more factors secreted by a glioblastoma cell [line] that have the following characteristics:

a) incubation of the composition with APCs presenting an antigen, and subsequent exposure of the incubated APCs to T cells specific for the antigen, induces the T cells to undergo anergy or apoptosis;

b) a molecular weight greater than about 40 kDa;

c) ability to bind to anion, but not cation exchange columns;

d) maintain an ability to induce T cells to undergo anergy or apoptosis under the conditions of a) within the pH range of 2 to 11, following heat exposure up to about 56° C, and following immunoprecipitation of TGF- ß1, TGF- ß2, TGF- ß3, IL-6, calcitonin gene related peptide (CGRP), and M-CSF from the composition; and

e) loses the ability to induce T cells to undergo anergy or apoptosis under the conditions of a) following heat exposure above 56° C, or after exposure to trypsin.

19. (Reiterated) The immunosuppressive composition of claim 18, wherein incubation of the composition with an effective amount of monocytes, dendrites, and B cells causes effects comprising the following:

a) decreased expression of MHC class II antigens and CD 80/86 on the surface of the monocytes and the dendrites, but no effect on the expression of MHC class II antigens and CD 80/86 on the B cells;

b) increased expression of IL-10 in monocytes and dendrites; and

c) decreased the expression of IL-12 in monocytes and dendrites.

20. (Amended) A method for enhancing tolerance in a host mammal to an allogenic donor graft, comprising:

[providing mammalian APCs from a donor mammal;]

exposing [the] APCs obtained from a donor mammal to a therapeutically effective amount of a composition secreted by a glioblastoma cell, wherein the composition is effective to induce the APCs obtained from the donor mammal to secrete one or more factors that selectively inhibit clonal proliferation of a T cell that specifically recognizes an allogenic antigen presented by the APCs obtained from the donor mammal; and

administering a therapeutically effective dose of the APCs obtained from a donor mammal that have been exposed to the therapeutically effective amount of the composition secreted by the glioblastoma cell to the host mammal to inhibit recognition of the allogenic antigen by the host mammal by inhibiting the clonal proliferation of the T cell of the host mammal in response to presentation of the allogenic antigen by the APCs.

21. (Amended) The method of claim 20, wherein the allogenic antigen is an antigen from the allogenic donor graft.

22. (Amended) The method of claim 20, wherein [providing] obtaining the donor mammalian APCs comprises specifically isolating or purifying APCs that recognize the allogenic antigen in the donor graft.

23. (Amended) The method of claim 22, wherein the APCs are [isolated] obtained from a source selected from the group consisting of an organ, tissue, bone marrow and blood of the donor mammal.

24. (Amended) A method for enhancing tolerance in a host mammal to an autoantigen, comprising:

[isolating mammalian] obtaining APCs from the host mammal, wherein the APCs present an antigen to T cells which recognize the antigen and undergo a specific clonal proliferation to induce an immune response against the antigen;

culturing the APCs ex vivo in an effective amount of a composition secreted by a glioblastoma cell[, the] in an amount [being] effective to induce the APCs to secrete one or more factors that selectively inhibit the specific clonal proliferation of the T cells that recognize the autoantigen presented by the APCs; and

administering a therapeutically sufficient dose of the APCs to the host mammal to inhibit recognition of the autoantigen by the host mammal.

25. (Amended) The method of claim 2, wherein introducing the APCs into the subject comprises administering the APCs by a route selected from the group consisting of intravenous, subcutaneous, intramuscular, and intraperitoneal administration.

26. (Reiterated) The composition of claim 18 for use as a medicament.

27. (Amended) The composition of clam 18 for use in a method of treating an immune mediated disease, comprising administering the composition to [a patient] the subject [said composition].

28. (Reiterated) A method of inhibiting an immune response to one more selected antigens comprising contacting an APC with the composition of claim 18.

29. (Reiterated) A method of making an immunosuppressive composition for use in suppressing an immune response to an antigen, comprising incubating a supernatant

harvested from a glioblastoma cell culture and the antigen with an APC, thereby producing an immunosuppressive composition that includes the APC.

31. (Amended) The method of claim[s] 29, further comprising combining the immunosuppressive composition with a pharmaceutical carrier.

32. (Reiterated) The composition obtained by the method of claim 29.

32. (Reiterated) The method of claim 29, further comprising purifying the APC to produce a substantially pure APC composition prior to incubating with the glioblastoma cell culture supernatant.

33. (Reiterated) The method of claim 29, further comprising purifying the APC to produce a substantially pure APC composition after incubating with the glioblastoma cell culture supernatant.

We claim:

1. A method of specifically inhibiting an immune response to one or more selected antigens comprising:

a) providing antigen presenting cells (APCs) that present an antigen against which selective inhibition of an immune response is desired;

b) incubating the APCs with an immunosuppressive composition comprising one or more factors secreted by a glioblastoma cell line.

2. The method of claim 1, further comprising

c) introducing the APCs into a subject in need of a reduced immune response to the antigen, in a therapeutically effective amount sufficient to selectively inhibit the immune response of the subject to the antigen.

3. The method of claim 1, wherein providing the APCs comprises obtaining APCs from a transplant donor, wherein the APCs express a transplant antigen.

4. The method of claim 1, wherein providing the APCs comprises obtaining APCs from the subject, wherein the APCs present an autoantigenic antigen.

5. The method of claim 5, wherein providing the APCs comprises incubating the APCs with autoantigenic peptides, in an amount effective to cause the APCs to present the autoantigenic peptide.

6. The method of claim 1, wherein the method inhibits the immune response by inducing apoptosis and/or anergy in T cells specific for the selected antigens.

7. The method of claim 2, wherein the APCs are obtained from a donor other than a subject, and the selected antigens are donor-specific antigens present on an allogenic graft.

8. The method of claim 2, wherein the APCs are obtained from a donor of an allogenic graft that is transplanted to the subject, and the introducing step comprises administering a sufficient dose of the incubated cells to the subject to specifically inhibit the subject's immune response against an antigen presented by the APCs of the donor.

9. The method of claim 1 wherein the selected antigen is an autoantigenic protein of an autoimmune disease.

10. The method of claim 8, wherein the providing step comprises isolating APCs from a subject suffering from an autoimmune disease, and repetitively exposing the isolated APCs to one or more peptide fragments of the autoantigenic protein of the autoimmune disease, and the introducing step comprises administering a therapeutically effective amount of the APCs to the subject.

11. The method of claim 10, wherein the autoimmune disease is selected from the group consisting of multiple sclerosis (MS), rheumatoid arthritis (RA), myasthenia gravis (MG), systemic lupus erythematosus (SLE), and insulin dependent diabetes mellitus (IDDM).

12. The method of claim 10, wherein the autoantigenic protein is selected from the group consisting of myelin basic protein (MBP), type II collagen, acetyl choline receptor (AcChoR), nuclear proteins, and pancreatic islet cell antigens.

13. The method of claim 1, wherein the APCs are selected from the group consisting of monocytes, macrophages, and dendritic cells.

14. The method of claim 13, wherein the APCs comprise monocytes.

15. The method of claim 8, wherein the APCs comprise monocytes isolated from the donor's blood.

16. The method of claim 9, wherein the APCs comprise monocytes isolated from the subject's blood.

17. The method of claim 1, wherein the glioblastoma line is selected from the group consisting of SNB 19, U251 A172, A1207, A1235, A2781, U87 MG, U138 MG and U373 MG.

18. A purified immunosuppressive composition for the reduction of an immune response to one or more selected antigens, the composition comprising one or more factors secreted by a glioblastoma cell line that have the following characteristics:

a) incubation of the composition with APCs presenting an antigen, and subsequent exposure of the incubated APCs to T cells specific for the antigen, induces the T cells to undergo anergy or apoptosis;

b) a molecular weight greater than about 40 kDa;

c) ability to bind to anion, but not cation exchange columns;

d) maintain an ability to induce T cells to undergo anergy or apoptosis under the conditions of a) within the pH range of 2 to 11, following heat exposure up to about 56° C, and following immunoprecipitation of TGF- ß1, TGF- ß2, TGF- ß3, IL-6, calcitonin gene related peptide (CGRP), and M-CSF from the composition; and

e) loses the ability to induce T cells to undergo anergy or apoptosis under the conditions of a) following heat exposure above 56° C, or after exposure to trypsin.

19. The immunosuppressive composition of claim 18, wherein incubation of the composition with an effective amount of monocytes, dendrites, and B cells causes effects comprising the following:

a) decreased expression of MHC class II antigens and CD 80/86 on the surface of the monocytes and the dendrites, but no effect on the expression of MHC class II antigens and CD 80/86 on the B cells;

b) increased expression of IL-10 in monocytes and dendrites; and

c) decreased the expression of IL-12 in monocytes and dendrites.

20. A method for enhancing tolerance in a host mammal to an allogenic donor graft, comprising:

providing mammalian APCs from a donor mammal;

exposing the APCs to a therapeutically effective amount of a composition secreted by a glioblastoma cell, wherein the composition is effective to induce the APCs to secrete one or more factors that selectively inhibit clonal proliferation of a T cell that specifically recognizes an allogenic antigen presented by the APCs; and

administering a therapeutically effective dose of the APCs to the host mammal to inhibit recognition of the allogenic antigen by the host mammal by inhibiting the clonal proliferation of the T cell of the host mammal in response to presentation of the allogenic antigen by the APCs.

21. The method of claim 20, wherein the allogenic antigen is an antigen from the donor graft.

22. The method of claim 20, wherein providing the mammalian APCs comprises specifically isolating APCs that recognize the allogenic antigen in the donor graft.

23. The method of claim 22, wherein the APCs are isolated from a source selected from the group consisting of an organ, tissue, bone marrow and blood of the donor mammal.

24. A method for enhancing tolerance in a host mammal to an autoantigen, comprising:

isolating mammalian APCs from the host mammal, wherein the APCs present an antigen to T cells which recognize the antigen and undergo a specific clonal proliferation to induce an immune response against the antigen;

culturing the APCs in an effective amount of a composition secreted by a glioblastoma cell, the amount being effective to induce the APCs to secrete one or more factors that selectively inhibit the specific clonal proliferation of the T cells that recognize the autoantigen presented by the APCs; and

administering a therapeutically sufficient dose of the APCs to the host mammal to inhibit recognition of the autoantigen by the host mammal.

25. The method of claim 2, wherein introducing the APCs comprises administering the APCs by a route selected from the group consisting of intravenous, subcutaneous, intramuscular, and intraperitoneal administration.

26. The composition of claim 18 for use as a medicament.

27. The composition of clam 18 for use in a method of treating an immune mediated disease, comprising administering to a patient said composition.

28. A method of inhibiting an immune response to one more selected antigens comprising contacting an APC with the composition of claim 18.

29. A method of making an immunosuppressive composition for suppressing an immune response to an antigen, comprising incubating a supernatant harvested from a glioblastoma cell culture and the antigen with an APC, thereby producing an immunosuppressive composition that includes the APC.

31. The method of claims 29, further comprising combining the immunosuppressive composition with a pharmaceutical carrier.

32. The composition obtained by the method of claim 29.

32. The method of claim 29, further comprising purifying the APC to produce a substantially pure APC composition prior to incubating with the glioblastoma cell culture supernatant.

33. The method of claim 29, further comprising purifying the APC to produce a substantially pure APC composition after incubating with the glioblastoma cell culture supernatant.